Methods: 33 pairs samples were collected from two clinical studies, assessing anti-malarial drug resistance in the Chittagong Hill tracts Bangladesh during 2004 and 2005. Blood samples were taken at Day 0 and at the day of recurrent parasitemia during 42 days follow-up and analyzed using PCR-RFLP and Tag-man real-time PCR assay.

Results: We found high parasite diversity in the population and polyclonal infection in the host using MSP-1 and GLURP loci. . MSP-1 allelic family K1 was positive in 19 (58%) with, 6 different fragments (150−280 bp)., MAD20 was detected in 25 (76%) with 8 different fragments (130-300 bp) and RO33 allele was detected in 21 (64%) samples with 4 different sizes. The region II of GLURP was present in all 33 (100%) samples with 6 different fragments (600−1100 bp). MAD-20 showed higher numbers of PCR positivity with higher numbers of allele. Molecular analysis of anti-malarial drug resistance marker such as pfcrt and pfmdr-1by PCR- RFLP showed high prevalence of mutan tpfcrt76T (90%) and (80%) of wild pfmdr1N86. Among the 33 paired samplespfmdr1represent 18.2% were heterozygous (N86Y) and 18.2% were mutant (86Y).

In the pre-treatment isolates 6 (18%) were CVMNK haplotype and all mixed with SVMNT haplotype. A total of 5 (83%) out of 6 were mixed with CVIET haplotype. In all 33 pre-treatment isolates SVMNT haplotype and 32 of them (97%) CVIET haplotype. In the post-treatment samples 10 (30%) isolates were CVMNK haplotype, 30 (91%) SVMNT haplotype and 24 (73%) CVIET haplotype, all of them mixed with SVMNT haplotype.

Conclusion: Genotype using antigenic polymorphic and anti-malarial drug resistance markers can be limit misclassification of recurrent parasitemia Analysis of K76T point mutation by PCR-RFLP and real-time PCR showed that both methods are equally proficient in detecting K76 and 76T alleles but Taq-man multiplex real-time is significantly better in detecting both alleles K76 and K76T (uncorrected X2 = 4.12, P = .042).

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Cultivation and molecular characterization of blastocystis spp. by polymerase chain reaction

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Background: Blastocystis *is* often reported as a causative agent of chronic diarrhoea in Pakistan. The objective of this study was isolation and culturing of Blastocystis spp obtained from diarrhoeal patients and poultry by using different cultural media. Molecular identification was done by PCR.

Methods: Three hundred and fifty fecal samples were collected from patients with gastrointestinal symptoms and their clinical histories were recorded. Direct microscopy and concentration method were used for the detection of parasites and Kinyoun method for Cryptosporidium. Three hundred and fifty poultry faecal samples were also collected and Blastocystis spp was detected by direct microscopy. Positive samples of Blastocystis spp were cultured on Jone's' medium supplemented with 10% horse serum and Locke-

Results: Results indicated that out of 350 faecal samples parasites were present in 40% of cases. Blastocystitis spp was present in 48% followed by *Entamoeba histolytica* (24%), Cryptosporidium spp (15%), *Giardia lamblia* (12%), *Ascaris lumbricoides* (1%). Single pathogen infection was present in 80% and in 20% of the cases infection was in combination with other parasites. The most frequent symptoms were abdominal pain in 50% of the cases, diarrhoea in 26%, fever in 16% and vomiting in 8% of the cases.

Out of 350 poultry samples (21%) were positive for Blastocystis spp. 50 positive samples of Blastocystis spp. from patients and 20 poultry samples cultured in vitro on Jone's media indicated no growth of *Blastocystis* spp. while on Locke-egg (LE) medium, heavy growth was obtained in all samples positive for Blastocystis on direct microscopy. After DNA extraction PCR was positive for most of the samples.

Conclusion: Blastocystis was present in chronic diarrheal patients either alone or in combination with other parasites. Detection of Blastocystis was greater by culture on Locke-egg media as compared to direct smear and PCR gave good results after DNA extraction of Blastocystis.

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Evaluation of in vitro anti-leishmanial activities of curcumin and its derivatives "gallium curcumin, indium curcumin and diacetylecurcumin"

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Background: *Leishmania* species are intracellular parasitic hemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrates' hosts. Curcumin is the active ingredient in the herbal remedy and dietary spice turmeric (*curcuma longa* linn). Curcumin was identified to be responsible for most of the biological effects of turmeric.

Methods: *Leishmania major* promastigotes were cultivated in RPMI 1640 and each well of micro plate was filled with a final concentration of 4×106 parasites/ml (100μl) of culture medium and after the incubation period, the test agents including curcumin, indium curcumin (In(CUR)3), diacetylcurcumin (DAC) and gallium curcumin (Ga(CUR)3) were added. Negative control only received RPMI medium, and the positive control contained varying concentrations of standard anti-leishmanial compound, amphotericin B. MTT solution was added to each well and incubated at 25°C for 72 hours. Afterward, isopropanol was added for solving the formazan crystals. Finally, the plates were read with an ELISA reader using 540 nm as test wavelength and 630 nm as the reference wavelength.

Results: The IC50 values for curcumin, Ga(CUR)3, In(CUR)3, DAC and amphotericine B were 38μg/ml, 32μg/ml, 26μg/ml, 52μg/ml